EasyPrep™ Gel Extraction and PCR Cleaning Manual

Catalog#: DC01-01, DC01-02

DC02-01, DC02-02

For research use only

(March 2013)

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Introduction

EasyPrep™ Gel Extraction and PCR Cleaning kit provide a fast and reliable method to recover DNA from agarose gels, and to purify DNA fragment from PCR, RFLP, phosphorylation, labeling and any other enzymatic reactions. DNA binds to the membrane in the presence of high concentration of salt. The other components such as nucleotides, enzymes, salts and dyes pass through the membrane and further washed away by Washing Buffer. Pure DNA is eluted with Elution Buffer (Tris buffer, 10 mM, pH8.5) or H_2O. DNA fragments from 200 bp to 20 kb can be purified using the DNA mini binding column with up to 70% recovery rate for gel extraction and 90% for PCR cleaning.

Binding Buffers

EasyPrep™ Gel Extraction kit and PCR Cleaning kit contains same DNA binding columns but different binding buffers to optimize the DNA recovery from each application.

- Buffer GB in the EasyPrep™ Gel Extraction kit solubilizes the agarose gel slices and enables the DNA binding to the membrane. It can also be used for PCR cleaning. The phenol red in the GB buffer is used as pH indicator. The pH of GB/solubilized agarose gel slice mixture is important for DNA binding. In case the color of GB/ solubilized agarose gel slice mixture turns from orange to pink, add a couple drops of 2.5 M Potassium Acetate or Sodium Acetate (pH 5.0) to change the color back.

- Buffer PB in the EasyPrep™ PCR Cleaning kit enables the DNA binding of single– or double-stranded PCR fragments (100bp to 10kb) to the membrane. Primers in the mixture will not bind to the membrane well and pass through the membrane, will be further removed by washing step. The kit can also be used for DNA purification from other enzymatic reactions, such as restriction enzyme reaction, cDNA synthesis or chromosome immune-precipitations (CHIP).

Kit Contents

1. Gel extraction kit

Catalog No.	**DC3511-01**	**DC3511-02**
New Catalog No.	**DC01-01**	**DC01-02**
DNA mini Columns	50	250
Buffer GB*	30 ml	130 ml
Wash Buffer Concentrate	12 ml	54 ml
Elution Buffer	10 ml	20 ml
2ml Collection Tubes	50	250
User Manual	1	1

* Buffer GB contains chaotropic salts. Wear gloves and use protective eyewear when handling this solution. Buffer GB may form precipitates under cool ambient condition. Warm up the buffer at 37^{o}C to dissolve before use.

2. PCR cleaning kit

Catalog No.	**DC3514-01**	**DC3514-02**
New Catalog No.	**DC02-01**	**DC02-02**
DNA mini Columns	50	250
Buffer PB	25 ml	125 ml
Wash Buffer Concentrate	12 ml	54 ml
Elution Buffer	10 ml	20 ml
2ml Collection Tubes	50	250
User Manual	1	1

Storage and Stability

All components can be stored at room temperature. The shelf life of the kit is guaranteed for 1 year from the date of purchasing.

Before Start

Prepare all components and get all necessary materials ready by examining this instruction booklet and become familiar with each steps.

Important:

- Add 96-100% ethanol to DNA Wash Buffer before use:
  - DC01-01/DC02-01: 48 ml
  - DC01-02/DC02-02: 216 ml
- A gel slice of 100 mg equals to a volume of 100 µl.

Materials supplied by users

- Tabletop microcentrifuge and 1.5 ml microtubes.
- 50-60^{o}C water bath (for gel extraction).
- Vacuum manifold if use vacuum Manual.
- Isopropanol and 70% ethanol

Perform all steps including centrifugation at room temperature!

Limited use and warranty

This product is warranted to perform as described in its labeling and in Bioland's literature when used in accordance with instructions. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Bioland. Bioland's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Bioland, to replace the products, Bioland shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

EasyPrep™ Gel Extraction protocol

(Centrifuge method)

1. **Excise the DNA fragment form the agarose gel and weigh it in a 1.5 ml microtube** (A gel slice of 100 mg approximately equals to 100 µl). **Add 3 gel volume Buffer GB and incubate the mixture at 50-60°C for 8 min with mixing the tube by tapping the tube bottom every 2-3 min till the gel has melted completely.**
2. **Add 1 gel volume Isopropanol to the mixture and mix.**
3. **Transfer up to 750 µl of the mixture to a DNA mini column with a collection tube. Centrifuge at 11,000 rpm for 30-60s. Discard the flowthrough and put the column back to the collection tube.** Repeat this step to process if there is remaining mixture.
4. **Add 750 µl DNA Wash Buffer to the column and centrifuge at 11,000 rpm for 1 min at room temperature. Discard the flow through.** Ensure that ethanol has been added to DNA Wash Buffer before use. For salt-sensitive applications, let the column stand with added washing buffer for 2-5 min, then centrifuge. This will reduce the salt in the DNA.
5. **Centrifuge the empty column at 11,000 rpm for 1 min to remove the residual ethanol.** It is critical to remove residual ethanol for optimal elution. Residual ethanol may interfere subsequent applications.
6. **Place the column in a clean 1.5 ml micocentrifuge tube. Add 20 -35 µl Elution Buffer or H_2O (pH7.5-8.5) to the column. Incubate at room temperature for 1 min. Centrifuge at 11,000 rpm for 1 min to elute the DNA.** If it's a large fragment (≥10 kb), pre-warm the elution buffer to 65°C will increase the yield.
7. Optional: **Re-apply the eluate to the column, elute one more time.** This will increase DNA yield.

EasyPrep™ Gel Extraction protocol

(Vacuum method)

1. **Excise the DNA fragment form the agarose gel and weigh it in a 1.5 ml microtube (A gel slice of 100 mg approximately equals to 100 µl). Add 3 gel volume Buffer GB and incubate the mixture at 50-60°C for 8 min with mixing the tube by tapping the tube bottom every 2 min till the gel has melted completely.**
2. **Add 1 gel volume Isopropanol to the mixture and mix.**
3. **Prepare the vacuum manifold according to manufacturer's instructions. Attach the spin column to the manifold.**
4. **Transfer up to 750 µl DNA/Buffer GC mixture to a DNA mini column. Switch on vacuum source to draw the solution through the columns completely, and then switch off vacuum source.**
5. **Add 750 µl DNA Wash Buffer to the column. Switch on vacuum source to draw the solution through the columns completely and then switch off vacuum source.** Ensure that ethanol has been added to DNA Wash Buffer before use. For salt-sensitive applications, let the column stand with added washing buffer for 2-5 min, then centrifuge.
6. **Transfer the DNA columns to 2 ml collection tubes. Centrifuge at 11,000 rpm for 1 min .** It is critical to remove residual ethanol for optimal elution. Residual ethanol may interfere subsequent applications.
7. **Place the column in a clean 1.5 ml micocentrifuge tube. Add 20 -35 µl Elution Buffer or H_2O (pH7.5-8.5) to the column. Incubate at room temperature for 1 min. Centrifuge at 11,000 rpm for 1 min to elute the DNA.** If it's a large fragment (≥10 kb), pre-warm the elution buffer to 65°C will increase the yield.
8. Optional: **Re-apply the eluate to the column, elute one more time.** This will increase DNA yield.

EasyPrep™ PCR Cleaning protocol

(Centrifuge method)

1. **Add 5 volume Buffer PB to the PCR reaction and mix well.** (i.e. add 250 µl PB to 50 µl PCR reaction). **Briefly spin the tube to collect any drops from the inside wall and tube lid.** It's not necessary to remove mineral oil.

2. **Transfer up to 750 µl of the mixture to a DNA mini column with a collection tube. Centrifuge at 11,000 rpm for 1 min. Discard the flowthrough and put the column back to the collection tube.** Repeat this step to process if there is remaining mixture.

3. **Add 750 µl DNA Wash Buffer to the column and centrifuge at 11,000 rpm for 1 min at room temperature. Discard the flow through.** Ensure that ethanol has been added to DNA Wash Buffer before use.

4. **Centrifuge the empty column at 11,000 rpm for 1 min to remove the residual ethanol.** It is critical to remove residual ethanol for optimal elution. Residual ethanol may interfere subsequent applications.

5. **Place the column in a clean 1.5 ml micocentrifuge tube. Add 20 -35 µl Elution Buffer or H_2O (pH7.5-8.5) to the column. Incubate at room temperature for 1 min. Centrifuge at 11,000 rpm for 1 min to elute the DNA.** If it's a large fragment (≥10 kb), pre-warm the elution buffer to 65°C will increase the yield.

6. Optional: **Re-apply the eluate to the column, elute one more time.** This will increase DNA yield.

EasyPrep™ PCR Cleaning protocol

(Vacuum method)

1. **Add 5 volume Buffer PB to the PCR reaction and mix well.** (i.e. add 250 µl PB to 50 µl PCR reaction). **Briefly spin the tube to collect any drops from the inside wall and tube lid.** It's not necessary to remove mineral oil.
2. **Prepare the vacuum manifold according to manufacturer's instructions. Attach the spin column to the manifold.**
3. **Transfer up to 750 µl DNA/Buffer GC mixture to a DNA mini column. Switch on vacuum source to draw the solution through the columns completely, and then switch off vacuum source.**
4. **Add 750 µl DNA Wash Buffer to the column. Switch on vacuum source to draw the solution through the columns completely and then switch off vacuum source.** Ensure that ethanol has been added to DNA Wash Buffer before use.
5. **Transfer the DNA columns to 2 ml collection tubes. Centrifuge at 11,000 rpm for 1 min .** It is critical to remove residual ethanol for optimal elution. Residual ethanol may interfere subsequent applications.
6. **Place the column in a clean 1.5 ml micocentrifuge tube. Add 20 -35 µl Elution Buffer or H_2O (pH7.5-8.5) to the column. Incubate at room temperature for 1 min. Centrifuge at 11,000 rpm for 1 min to elute the DNA.** If it's a large fragment (≥10 kb), pre-warm the elution buffer to 65^{o}C will increase the yield.
7. Optional: **Re-apply the eluate to the column, elute one more time.** This will increase DNA yield.

Trouble shooting Guide

Problem	Possible reason	Suggested improvement
Low DNA yield	Not enough buffer GC	Determine the volume of Buffer GC to be used correctly as instructed. For PCR products less than 200 bp, add 2 volume Buffer GC.
	Agarose gel doesn't melt completely	Make sure to set the water bath to 55-60^{0}C to allow gel to melt completely. Add more buffer C3 if necessary.
No DNA yield	Forgot to add ethanol to DNA Wash Buffer	Add absolute ethanol to DNA Wash Buffer as instructed before use.
DNA Sample floats out of well while loading agarose gel	Ethanol was not completely removed from the column following wash step	After the wash step, centrifuge the empty column at top speed for 1 min. Repeat once.

More EasyPrep™ Products

New Cat#	Old Cat#	Description	Size
PD01-01	PD1211-01	Plasmid Miniprep kit	50 preps
PD01-02	PD1211-02	Plasmid Miniprep kit	250 preps
PD02-01	PD1213-01	Plasmid Miniprep-II kit	50 preps
PD02-02	PD1213-02	Plasmid Miniprep-II kit	250 preps
PD03-01	PD1411-01	Plasmid Midiprep kit	10 preps
PD03-02	PD1411-02	Plasmid Midiprep kit	25 preps
PD03-03	PD1411-03	Plasmid Midiprep kit	50 preps
PD04-01	PD1412-01	Plasmid Midiprep-II kit	10 preps
PD04-02	PD1412-02	Plasmid Midiprep-II kit	25 preps
PD03-11	PD1413-01	ezFilter Plasmid Midiprep kit	10 preps
PD03-12	PD1413-02	ezFilter Plasmid Midiprep kit	25 preps
PD03-13	PD1413-03	ezFilter Plasmid Midiprep kit	50 preps
PD04-11	PD1416-01	ezFilter Plasmid Midiprep-II kit	10 preps
PD04-12	PD1416-02	ezFilter Plasmid Midiprep-II kit	25 preps
PD05-01	PD1511-01	Plasmid Maxiprep kit	10 preps
PD05-02	PD1511-02	Plasmid Maxiprep kit	25 preps
PD05-11	PD1512-01	ezFilter Plasmid Maxiprep kit	10 preps
PD05-12	PD1512-02	ezFilter Plasmid Maxiprep kit	25 preps

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